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Evolutionary Speed of Enzymes Functional Surfaces and their Relationship with Metabolic Fluxes in Networks of Central Carbon Metabolism of Bacteria

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Understanding how enzymes emerge, interact, and work together in biochemical networks is a challenging task. The concentrations of enzymes, substrates, products, and their time-dependent changes, the organization of structures of enzymes involved, as well as their evolutionary histories should all be integrated for an improved comprehensive understanding of the systems behavior of the biochemical networks. In this study, we explore the relationship between enzyme structures, enzyme functions, metabolite concentrations and fluxes in the glycolysis and pentose-phosphate pathways, which form the central carbon metabolism of E. coli. As large amount of structural information is available for most components of these metabolic pathways, we have estimated the evolutionary rates of individual functional surface of enzymes in these pathways. To separate selection pressure due to enzyme function from other selection pressures such as structural integrity, folding stability, and folding kinetics, we have estimated the substitution rates of residues located in the functional pockets of these enzyme structures, including residues involved in the catalytic reaction, and residues in the surrounding area participating in the accommodation of both substrates and products. These are then summarized into an overall index of evolutionary speed for enzyme functional surfaces. In conjunction of stoichiometry based flux-balance analysis, we have also simulated time-dependent evolution of metabolite concentrations and fluxes based on ordinary differential equation models and currently available enzyme kinetic parameters. We discuss our findings in overall relationship of evolutionary speed of enzyme function and importance of individual enzyme measured by flux balance and kinetic flow analysis, in the global context of the central carbon metabolism of E coli.

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Functional Assignment of Hypothetical Proteins from Protein-Protein Interaction Networks

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Determining protein functions on proteomic scale is a challenging problem. Almost 8 million non-redundant sequences of proteins are determined from more than 2000 species. The number of protein sequences is doubling every 28 months, however functional assignment for new discovered proteins lacks behind. Computational methods based on sequence or structure similarities, clustering of co-regulated genes, and phylogenetic profile mapping are reliable for about eighty percent of all protein sequences. For the remaining twenty percent the functional assignment fails. Elucidation of protein function and localization in the cell for uncharacterized proteins will be inevitable to describe biological processes on a systems level. Here we present a high-throughput experimental method to assign protein function of uncharacterized proteins based on protein-protein interaction networks. We demonstrate that it is possible to generate a whole genome protein-protein interaction map for one protein in a day experiment. For this we exploit a recently developed microfluidic chip. The highly integrated device is a combination of rapid prototyping in polydimethylsiloxane and DNA microarray technology. One microfluidic device allows in vitro expression of thousands of proteins and can perform 4K high affinity measurements in parallel. In order to be able to characterize unknown proteins by protein-protein interactions on a proteomic scale we constructed a cDNA library of the Streptoccocus pneumonia (SP) proteome. After optimization 2/3 of the SP proteome could be expressed on chip and used for high throughput screening. For functional assignment of 25 highly conserved hypothetical or hypothetical proteins specific for the SP organism we performed 35,000 affinity measurements. The resulting interaction network and its network parameters are used to categorize the hypothetical proteins to common functional classes.

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Hydration Pressure Increases in Heat-Damaged Skin:osmotic-Stress and High-Resolution Magnetic Resonance Data Implicate Local Supramolecular Mechanisms

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Local forces driving interstitial-fluid transfer affect burns; heat-ablation and remodeling therapies; hemodynamics; and wound healing. We examined hydration/dehydration mechanisms after heating skin ex vivo at 40, 50, and 60°C to induce ''denaturing'' transitions in three main structures of the interstitial matrix: hyaluronic acid, cells, and collagen.
Hydration pressure (HP), swelling rates, water activity, acid-binding capacity (at pH~7.4) and transverse nuclear-relaxation rate of solvent-water protons (1/T2) were compared using osmotic-stress, gravidometric, saturation vapor-pressure, potentiometric, and magnetic resonance imaging (μMRI, 40μm /pixel resolution) analyses, respectively. Mean HP values were 89, 100, 79, 108 at 4°C and 50, 62, 91, 162 mmHg at 37°C in nonheated control, 40, 50, and 60°C samples, respectively. Swelling kinetic energy increased 1.1, 4.5, and 6.9-fold relative to controls. Acid-binding and water activity increased by 0.3 and 0.4 pH units and 0.020 and 0.033 (at 75% hydration) in 50 and 60°C samples, while in 40°C samples, values remained close to controls'. μMRI changes were obvious only in 60°C samples with 1/T2 gradients (from T2-map contrast), and average 1/T2 in the central dermis decreased 0.1 intensity units/μm and 66%, respectively.
HP and acid-binding data indicate increases in the total solvent-exposed area and, with mutually consistent 1/T2 decreases and water-activity increases, demonstrate qualitative changes in its average hydration properties. Quantitative differences among control and preheated samples and between hydration parameters obtained at 4 versus 37°C confirm temperature-dependent supramolecular reorganization following damage. Together, results provide strong evidence that multiscale interactions among matrix components, independent of vascular, hormonal and neural controls, change key interstitium fluid-transfer properties with heat-damage.

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Reactivity of Human Holocarboxylase Synthetase Toward Biotin-Accepting Substrates

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Human Holocarboxylase Synthetase (HCS) catalyzes biotin transfer to carboxylases in a two-step reaction in which the activated biotin, bio-5′-AMP, is first formed from substrates biotin and ATP. The biotin is then transferred to a single lysine residue on the carboxylase. The five substrates for HCS, including acetyl-CoA carboxylases 1 and 2, pyruvate carboxylase, 3-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase, play critical roles in metabolism. Two isoforms of HCS differ at the amino-terminus by 57 amino acids, and their specificity towards the substrates is unknown. The biochemistry of HCS function has been investigated by characterizing the basic properties of the two isoforms and their interactions with the five acceptor-protein substrates. Equilibrium sedimentation indicates that the proteins are monomers in their apo-forms and when bound to the adenylated intermediate. Steady state analysis of the overall reaction indicates that both isoforms possess similar behavior with respect to the small molecule substrates. In contrast, stopped flow fluorescence measurements of biotin transfer indicate that the HCS forms can exhibit distinct association rates with a single biotin acceptor. Moreover, the isoforms display preferential reactivity among the substrates. These results are consistent with a role for HCS N-terminus in acceptor substrate recognition, and suggest a role for HCS in dictating a hierarchy of biotin utilization by carboxylases.

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Biological Failure Model for Pancreatic β-Cell Dysfunctions

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Dysfunctions of pancreatic β-cells are one of the most critical features for both T1DM and T2DM. Although autoimmune attack is thought to be most common cause of T1DM, the detailed mechanisms of the progression from mild pancreatitis to severe β-cell dysfunctions are not fully understood. On